Supplementary Information

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Enhanced bacterial expression of several mammalian cytochrome P450s by codon optimization and chaperone coexpression

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Table S1. Oligonucleotides for the synthesis of native Synthon 1 of 27C1

Oligo name	Length	Sequence (5'->3')
27C1ns1-1	49	TAGGAGGTCATATGGCTGGTCCACGTAGTCTGGCCGCCATGCCGGGGCC
27C1ns1-2	55	CCGTCCCTGCAGAAGAACTCCGCCAGGTTGGCGAGGGTCCTCGGCCCCGGCATGG
27C1ns1-3	55	GTTCTTCTGCAGGGACGGCTTCAGCCGCATCCACGAGATCCAGCAGAAGCACACA
27C1ns1-4	55	ACCAAAGTGAGACTTGAAGATTTTTCCATATTCCCGTGTGTGCTTCTGCTGGATC
27C1ns1-5	55	GAAAAATCTTCAAGTCTCACTTTGGTCCTCAGTTTGTAGTATCTATTGCAGACCG
27C1ns1-6	55	TCCGCCCGGAGCACCTGAGCCACCATATCGCGGTCTGCAATAGATACTACAAACT
27C1ns1-7	55	TGCTCCGGGCGGAGGGCGCTGCGCCCCAGAGAGCCAACATGGAGTCCTGGCGGGA
27C1ns1-8	55	CGAGATGAGCCCGGTGGCTCTCCCCCGCAAGTCTCGGTACTCCCGCCAGGACTCC
27C1ns1-9	55	CACCGGGCTCATCTCGGCGGAGGGTGAACAGTGGCTCAAGATGAGAAGCGTATTG
27C1ns1-10	55	GCCACATCTTTCGGTTTCAGAATTCTTTGTCTCAATACGCTTCTCATCTTGAGCC
27C1ns1-11	55	TCTGAAACCGAAAGATGTGGCCATCTATTCTGGAGAAGTCAACCAAGTTATTGCT
27C1ns1-12	55	TCCTGAGGAGGTAGATTCTTTTAATTAAGTCAGCAATAACTTGGTTGACTTCTCC
27C1ns1-13	55	CTTAATTAAAAGAATCTACCTCCTCAGGAGCCAGGCAGAAGATGGAGAAACCGTG
27C1ns1-14	55	TTGAATATTTGAAGAAAAGATCATTGACATTGGTCACGGTTTCTCCATCTTCTGC
27C1ns1-15	55	CCAATGTCAATGATCTTTTCTTCAAATATTCAATGGAAGGAGTGGCCACCATCCT
27C1ns1-16	55	GGATGCTGTTTTCCAGGCAGCCCAAACGACTCTCATAAAGGATGGTGGCCACTCC
27C1ns1-17	55	TGCCTGGAAAACAGCATCCCACAGCTGACTGTGGAATACATCGAGGCCCTGGAGC
27C1ns1-18	55	GCGCCTGCATACATGGAGGTCTTGAACATGCTAAACATGAGCTCCAGGGCCTCGA
27C1ns1-19	55	TCCATGTATGCAGGCGCCATCCCCAGATGGCTTCGCCCCTTCATCCCAAAGCCCT
27C1ns1-20	55	TTGAAGAGTCCATCCCAGGACCTGCAGAATTCCCGCCAGGGCTTTGGGATGAAGG
27C1ns1-21	55	GTCCTGGGATGGACTCTTCAAATTCAGCCAAATTCATGTTGACAACAAGTTGAGG
27C1ns1-22	55	CCGGCCTCGGTCCATTTGGTACTGTATGTCCCTCAACTTGTTGTCAACATGAATT
27C1ns1-23	55	TGGACCGAGGCCGGAGGGTGAGCGGGGGACTTCTCACATACCTCTTCCTTAGCCA
27C1ns1-24	50	GGCGTAGATCTCCTGCAGCGTCAGAGCCTGGCTAAGGAAGAGGTATGTGA

Table S2. Oligonucleotides for the synthesis of native Synthon 2 of 27C1

Oligo name	Length	Sequence (5'->3')
27C1ns2-1	22	GCAGGAGATCTACGCCAACGTG
27C1ns2-2	55	ACGTCGTGTCGACGCCGGCCAGCAGCATCTCAGTCACGTTGGCGTAGATCTCCTG
27C1ns2-3	55	GGCGTCGACACGACGTCCTTCACCTTGTCTTGGACTGTGTACCTCCTGGCAAGGC
27C1ns2-4	55	CAATCTCCCGGTACACCGTCTGCTGCACTTCTGGGTGCCTTGCCAGGAGGTACAC
27C1ns2-5	55	GACGGTGTACCGGGAGATTGTGAAGAATTTAGGGGAAAGGCATGTTCCAACTGCA
27C1ns2-6	55	GAGCTCTGACCAGCGGGACCTTGGGGACATCAGCTGCAGTTGGAACATGCCTTTC
27C1ns2-7	55	CCCGCTGGTCAGAGCTCTCCTTAAGGAAACCCTGAGGCTGTTTCCAGTGCTGCCA
27C1ns2-8	55	AATAACCAGGTCTTCCTGGGTGACCCGGCCGTTCCCTGGCAGCACTGGAAACAGC
27C1ns2-9	55	TCACCCAGGAAGACCTGGTTATTGGCGGGTATCTGATTCCGAAAGGCACCCAGCT
27C1ns2-10	55	TCTCATCCTGGTACGATGTGGCATAGTGGCAAAGGGCCAGCTGGGTGCCTTTCGG
27C1ns2-11	55	GCCACATCGTACCAGGATGAGAACTTCCCTCGGGCCAAGGAGTTCCGGCCTGAGC
27C1ns2-12	55	ATTGTCAACTCTATCTAAGTCTCCTTTCCGCAGCCAGCGCTCAGGCCGGAACTCC
27C1ns2-13	55	GGAAAGGAGACTTAGATAGAGTTGACAATTTTGGATCCATCCCCTTTGGTCATGG
27C1ns2-14	55	TTCTGCAATTCTCCGCCCTATGCAGCTGCGAACCCCATGACCAAAGGGGATGGAT
27C1ns2-15	55	ATAGGGCGGAGAATTGCAGAACTGGAGATTCACCTCGTCGTGATCCAGTTGCTTC
27C1ns2-16	55	TTGGTCTGAGAAGATGTTTTGATCTCAAAATGTTGAAGCAACTGGATCACGACGA
27C1ns2-17	55	TTTGAGATCAAAACATCTTCTCAGACCAATGCTGTTCATGCAAAAACCCACGGGC
27C1ns2-18	55	CAAATCGCACGTGGATGGGCCCCCCTGGCGTCAGGAGCCCGTGGGTTTTTGCATG
27C1ns2-19	55	CCCATCCACGTGCGATTTGTTAACAGAAAGCACCATCATCACCATTAATCTAGAG
27C1ns2-20	32	TACCTCTAGATTAATGGTGATGATGGTGCTTT

Table S3. Oligonucleotides for the synthesis of optimized 2B1

Oligo name	Length	Sequence (5’->3’)
2B1-1	49	TAGGAGGTCATATGGCTCTGTTATTAGCAGTTTTTCTGGCGCTGCTGGT
2B1-2	50	GTGACCACGCACCAGTAACAGTAAGAAGCCAACCAGCAGCGCCAGAAAAA
2B1-3	50	CTGTTACTGGTGCGTGGTCACCCAAAATCTCGCGGTAATTTTCCACCAGG
2B1-4	50	AGTTGCCTAACAGCGGCAGTGGACGCGGACCTGGTGGAAAATTACCGCGA
2B1-5	50	TGCCGCTGTTAGGCAACTTATTACAGTTAGACCGTGGCGGTCTGTTAAAC
2B1-6	50	GCCGTATTTTTCACGTAATTGCATGAAAGAGTTTAACAGACCGCCACGGT
2B1-7	50	CATGCAATTACGTGAAAAATACGGCGATGTTTTTACCGTGCATTTAGGCC
2B1-8	50	CCACATAACATGACAACTGGACGTGGGCCTAAATGCACGGTAAAAACATC
2B1-9	50	ACGTCCAGTTGTCATGTTATGTGGCACCGACACCATTAAAGAAGCGTTAG
2B1-10	50	CCGCTGAAATCTTCTGCTTGGCCGACTAACGCTTCTTTAATGGTGTCGGT
2B1-11	50	CCAAGCAGAAGATTTCAGCGGCCGTGGTACGATCGCAGTGATCGAACCAA
2B1-12	50	GGCGAAGATAACACCATATTCCTTAAAGATTGGTTCGATCACTGCGATCG
2B1-13	50	TCTTTAAGGAATATGGTGTTATCTTCGCCAATGGCGAACGTTGGAAAGCC
2B1-14	50	TCGCGCATCGTTGCCAGAGAGAAGCGACGTAAGGCTTTCCAACGTTCGCC
2B1-15	50	TGGCAACGATGCGCGATTTCGGCATGGGTAAACGTAGCGTGGAAGAGCGT
2B1-16	50	TCCTCGACTAAGCATTGGGCCTCCTCTTGAATACGCTCTTCCACGCTACG
2B1-17	50	GCCCAATGCTTAGTCGAGGAGCTGCGCAAAAGCCAGGGCGCACCACTGGA
2B1-18	50	TATTGGCCGTAATACATTGAAACAGAAAGGTCGGATCCAGTGGTGCGCCC
2B1-19	50	CTTTCTGTTTCAATGTATTACGGCCAATATCATTTGCAGCATCGTGTTCG
2B1-20	50	TTGACGATCCGTGTAATCAAAACGCTCGCCGAACACGATGCTGCAAATGA
2B1-21	50	CGTTTTGATTACACGGATCGTCAATTCCTGCGTTTACTGGAACTGTTTTA
2B1-22	50	GCTGCTTAATAAGCTGAACGTGCGATAAAACAGTTCCAGTAAACGCAGGA
2B1-23	50	GCACGTTCAGCTTATTAAGCAGCTTTAGCAGCCAAGTTTTCGAGTTCTTT
2B1-24	50	CCGGGAAGTATTTCAGGAAGCCGCTAAAGAACTCGAAAACTTGGCTGCTA
2B1-25	50	GGCTTCCTGAAATACTTCCCGGGTGCCCACCGCCAGATTAGCAAGAATTT
2B1-26	50	TGGCCGATATAGTCCAGGATCTCCTGTAAATTCTTGCTAATCTGGCGGTG
2B1-27	50	GAGATCCTGGACTATATCGGCCATATTGTCGAGAAACACCGCGCGACCTT
2B1-28	50	GTATCGATGAAGTCACGTGGGGCGCTCGGGTCTAAGGTCGCGCGGTGTTT
2B1-29	50	CCCCACGTGACTTCATCGATACCTACCTGTTACGTATGGAGAAGGAAAAG
2B1-30	50	GGAACACGGTATGGTGATTGCTCTTTTCCTTCTCCATACGTAACAGGTAG
2B1-31	50	GCAATCACCATACCGTGTTCCATCACGAGAATCTGATGATCAGCCTGCTG
2B1-32	50	CGTTTCCGTGCCTGCGAAAAACAGAGACAGCAGGCTGATCATCAGATTCT
2B1-33	50	TCGCAGGCACGGAAACGTCTAGCACCACCTTACGCTATGGTTTCCTGCTG
2B1-34	50	TTCGGCCACGTGTGGGTACTTTAACATCAGCAGGAAACCATAGCGTAAGG
2B1-35	50	CCCACACGTGGCCGAAAAGGTTCAGAAGGAGATTGATCAGGTTATCGGCT
2B1-36	50	TCATCTAACGTCGGTAAGCGGTGAGAGCCGATAACCTGATCAATCTCCTT
2B1-37	50	CCGCTTACCGACGTTAGATGATCGTTCTAAGATGCCGTACACCGATGCAG
2B1-38	50	TAAATCGCTAAAACGTTGAATTTCGTGGATAACTGCATCGGTGTACGGCA
2B1-39	50	CCACGAAATTCAACGTTTTAGCGATTTAGTCCCGATTGGCGTTCCACATC
2B1-40	50	TAACCGCGGAACATGGTATCTTTCGTGACGCGATGTGGAACGCCAATCGG
2B1-41	50	AAAGATACCATGTTCCGCGGTTATTTACTGCCAAAAAACACGGAAGTCTA
2B1-42	50	GTGCAGGGCAGAGCTCAGAATTGGGTAGACTTCCGTGTTTTTTGGCAGTA
2B1-43	50	TGAGCTCTGCCCTGCACGATCCACAATATTTCGACCATCCAGATAGCTTC
2B1-44	50	ATCCAGGAAATGCTCTGGGTTGAAGCTATCTGGATGGTCGAAATATTGTG
2B1-45	50	AACCCAGAGCATTTCCTGGATGCAAACGGCGCACTGAAAAAATCTGAAGC
2B1-46	50	CGTTTGCCGGTGCTAAATGGCATGAAGGCTTCAGATTTTTTCAGTGCGCC
2B1-47	50	CCATTTAGCACCGGCAAACGTATTTGCCTGGGTGAGGGCATCGCGCGTAA
2B1-48	50	TGTAAAATCGTGGTGAAGAACAGGAACAGCTCATTACGCGCGATGCCCTC
2B1-49	50	TCCTGTTCTTCACCACGATTTTACAGAACTTCTCTGTCAGCTCTCATCTG
2B1-50	50	GGGTTAAGTCAATATCCTTCGGGGCCAGATGAGAGCTGACAGAGAAGTTC
2B1-51	50	CCCCGAAGGATATTGACTTAACCCCAAAAGAGAGCGGTATCGGCAAGATC
2B1-52	50	GCGCTGAAGCAAATCTGGTACGTTGGCGGGATCTTGCCGATACCGCTCTC
2B1-53	50	GTACCAGATTTGCTTCAGCGCACGCCATCATCACCACCATCACTAATCTA
2B1-54	32	TACCTCTAGATTAGTGATGGTGGTGATGATGG

Table S4. Oligonucleotides for site direct mutagenesis of 2S1 and 2U1 to modify N-terminal contiguous rare codons.

Constructs	Primers (5'–3')	
2S1C1	2S1C1F	GGGAAGCTGCCGCCAGGGCCAACGCCGCTACC
	2S1C1R	GGTAGCGGCGTTGGCCCTGGCGGCAGCTTCCC
2S1C2	2S1C2F	CCTCCTGCAGCTGCGTCCGGGGGCGCTGTATTC
	2S1C2R	GAATACAGCGCCCCCGGACGCAGCTGCAGGAGG
2U1C1	2U1C1F	GTAAGCTGCCGCCGGGGCCAACGCCGTGGCCTCTGG
	2U1C1R	CCAGAGGCCACGGCGTTGGCCCTGGCGGCAGCTTAC
2U1C2	2U1C2F	CTCCCTTCCTCCGTCGCCGTAGCTGGCTGAGC
	2U1C2R	GCTCAGCCAGCTACGGCGACGGAGGAAGGGAG
2U1C3	2U1C3F	CTGGCTGAGCAGCCGTACCCGCGCCGCAGGGATTG
	2U1C3R	CAATCCCTGCGGCGCGGGTACGGCTGCTCAGCCAG

Table S5. Oligonucleotides for N-terminal modifications of 2B1 and 2W1

		Primers
2W1 orignal	forward	TGCTCTGCCATATGGCTAAGAAAACGAGCTCTAAAGGTAAGCTGCCCCCGGGGCCTCGCCCGCTG
	reverse	GACTCTAGATTAGTGATGGTGATGATGGGGCCTGGGCACCGCACACAGG
2B1 orignal	forward	TGTTACTCCATATGGCTAAGAAAACGAGCTCTAAAGGTAAGCTGCCACCAGGACCTCGTCCCCTTCC
	reverse	TGCTCTAGATTAGTGGTGGTGGTGGTGCCGAGCTGAGAAGCAGATCTG
2B1 optimized	forward	TACTGTTACATATGGCTAAGAAAACGAGCTCTAAAGGTAAGCTGCCACCAGGTCCGCGTCCACTG
	reverse	TACCTCTAGATTAGTGATGGTGGTGATGATGG

Table S6. Nucleotide analysis of P450s with optimized or original codons. Estimated online using Optimizer (http://genomes.urv.es/OPTIMIZER/).

	CAI	%GC	%AT
27C1opt	0.562	53.3	46.7
27C1org	0.313	54.3	45.7
2W1opt	0.537	57.3	42.7
2W1orgt	0.342	68.9	31.1
2B1opt	0.554	49.2	50.8
2B1org	0.324	51.2	48.8

Fig.S1 Modifications introduced into P450 2B1 cDNA to optimize codon usage in *E. coli*. Top line, predicted amino acid sequence; middle line, original nucleotide sequence; lower line, nucleotide sequence optimized for *E. coli* expression.

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     M  A  L  L  L  A  V  F  L  A  L  L  V  G  F  L  L  L  L  V  R  G  H  P  K  S  R  G  N
ORI  ATGGCTCTGTTATTAGCAGTTTTTCTGGCTCTGTTAGTTGGTTTTCTGTTACTCTTAGTCCGTGGTCACCCAAAGTCCCGTGGCAAC   87
OPT  -----------------------------G---C-G-----C--CT-AC-GT-AC-G--G--------------A--T--C--T--T   87
     F  P  P  G  P  R  P  L  P  L  L  G  N  L  L  Q  L  D  R  G  G  L  L  N  S  F  M  Q  L
ORI  TTCCCACCAGGACCTCGTCCCCTTCCCCTCTTGGGGAACCTCCTGCAGTTGGACAGAGGGGGCCTCCTCAATTCCTTCATGCAGCTT   174
OPT  --T--------T--G-----A--G--G--G--A--C---T-AT-A-----A---C-T--C--T--GT-A--C--T--------AT-A   174
     R  E  K  Y  G  D  V  F  T  V  H  L  G  P  R  P  V  V  M  L  C  G  T  D  T  I  K  E  A
ORI  CGAGAAAAATATGGAGATGTGTTCACAGTACACCTGGGACCAAGGCCTGTGGTCATGCTATGTGGGACAGACACCATAAAGGAGGCT   261
OPT  --T--------C--C-----T--T--C--G--TT-A--C---C-T--A--T------T-------C--C--------T--A--A--G   261
     L  V  G  Q  A  E  D  F  S  G  R  G  T  I  A  V  I  E  P  I  F  K  E  Y  G  V  I  F  A
ORI  CTGGTGGGCCAAGCTGAGGATTTCTCTGGTCGGGGAACAATCGCTGTGATTGAGCCAATCTTCAAGGAATATGGTGTGATCTTTGCC   348
OPT  T-A--C--------A--A------AGC--C--T--T--G-----A-----C--A--------T--------------T-----C---   348
     N  G  E  R  W  K  A  L  R  R  F  S  L  A  T  M  R  D  F  G  M  G  K  R  S  V  E  E  R
ORI  AATGGGGAACGCTGGAAGGCCCTTCGGCGATTCTCTCTGGCTACCATGAGAGACTTTGGGATGGGAAAGAGGAGTGTGGAAGAACGG   435
OPT  -----C-----T-----A--T--A--T--C-----------A--G---C-C--T--C--C-----T--AC-T--C--------G--T   435
     I  Q  E  E  A  Q  C  L  V  E  E  L  R  K  S  Q  G  A  P  L  D  P  T  F  L  F  Q  C  I
ORI  ATTCAGGAGGAAGCCCAATGTTTGGTGGAGGAACTGCGGAAATCCCAGGGAGCCCCACTGGATCCCACCTTCCTCTTCCAGTGCATC   522
OPT  -----A-----G--------C--A--C-----G-----C---AG------C--A-----------G-----T--G--T--A--T--T   522
     T  A  N  I  I  C  S  I  V  F  G  E  R  F  D  Y  T  D  R  Q  F  L  R  L  L  E  L  F  Y
ORI  ACAGCCAACATCATCTGCTCCATTGTGTTTGGAGAGCGCTTTGACTACACAGACCGCCAGTTCCTACGCCTGTTGGAGCTGTTCTAC   609
OPT  --G-----T-----T---AG---C-----C--C-----T-----T-----G--T--T--A-----G--TT-AC----A-----T--T   609
     R  T  F  S  L  L  S  S  F  S  S  Q  V  F  E  F  F  S  G  F  L  K  Y  F  P  G  A  H  R
ORI  CGGACCTTTTCCCTCCTAAGTTCATTCTCCAGCCAGGTGTTTGAGTTCTTCTCTGGGTTCCTGAAATACTTTCCTGGTGCCCACAGA   696
OPT  --C--G--CAG-T-AT----CAGC--TAG------A--T--C--------TAGC--C--------------C--G---------C-C   696
     Q  I  S  K  N  L  Q  E  I  L  D  Y  I  G  H  I  V  E  K  H  R  A  T  L  D  P  S  A  P
ORI  CAAATCTCCAAAAACCTCCAGGAAATCCTCGATTACATTGGCCATATTGTGGAGAAGCACAGGGCCACCTTAGACCCAAGCGCTCCA   783
OPT  --G--TAG---G--TT-A-----G-----G--C--T--C-----------C-----A---C-C--G-----------G-----C---   783
     R  D  F  I  D  T  Y  L  L  R  M  E  K  E  K  S  N  H  H  T  V  F  H  H  E  N  L  M  I
ORI  CGAGACTTCATCGACACTTACCTTCTGCGCATGGAGAAGGAGAAGTCGAACCACCACACAGTGTTCCATCATGAGAACCTCATGATC   870
OPT  --T-----------T--C-----GT-A--T-----------A---AGC--T-----T--C-----------C-----T--G------   870
     S  L  L  S  L  F  F  A  G  T  E  T  S  S  T  T  L  R  Y  G  F  L  L  M  L  K  Y  P  H
ORI  TCCCTGCTCTCTCTCTTCTTTGCTGGCACTGAGACCAGCAGCACCACACTCCGCTATGGTTTCCTGCTGATGCTCAAGTACCCCCAT   957
OPT  AG------G-----G--T--C--A-----G--A--GTCT--------CT-A---------------------T-A--------A--C   957
     V  A  E  K  V  Q  K  E  I  D  Q  V  I  G  S  H  R  L  P  T  L  D  D  R  S  K  M  P  Y
ORI  GTCGCAGAGAAAGTCCAAAAGGAGATTGATCAGGTGATCGGCTCACACCGGCTACCAACCCTTGATGACCGCAGTAAAATGCCATAC   1044
OPT  --G--C--A--G--T--G-----------------T--------T-----CT----G--GT-A-----T--TTC---G-----G---   1044
     T  D  A  V  I  H  E  I  Q  R  F  S  D  L  V  P  I  G  V  P  H  R  V  T  K  D  T  M  F
ORI  ACTGATGCAGTTATCCATGAGATTCAGAGGTTTTCAGATCTTGTCCCTATTGGAGTACCACACAGAGTCACCAAAGACACCATGTTC   1131
OPT  --C--------------C--A-----AC-T---AGC---T-A-----G-----C--T-----TC-C-----G-----T---------   1131
     R  G  Y  L  L  P  K  N  T  E  V  Y  P  I  L  S  S  A  L  H  D  P  Q  Y  F  D  H  P  D
ORI  CGAGGGTACCTGCTTCCCAAGAACACTGAAGTGTACCCCATCCTGAGTTCAGCTCTCCATGACCCACAGTACTTTGACCACCCAGAC   1218
OPT  --C--T--TT-A--G--A--A-----G-----C-----A--T-----C--T--C--G--C--T-----A--T--C-----T-----T   1218
     S  F  N  P  E  H  F  L  D  A  N  G  A  L  K  K  S  E  A  F  M  P  F  S  T  G  K  R  I
ORI  AGCTTCAATCCTGAACACTTCCTGGATGCCAATGGGGCACTGAAAAAGAGTGAAGCTTTCATGCCCTTCTCCACAGGAAAGCGCATT   1305
OPT  --------C--A--G--T-----------A--C--C-----------ATC------C--------A--TAG---C--C--A--T---   1305
     C  L  G  E  G  I  A  R  N  E  L  F  L  F  F  T  T  I  L  Q  N  F  S  V  S  S  H  L  A
ORI  TGTCTTGGCGAAGGCATTGCCCGAAATGAATTGTTCCTCTTCTTCACCACCATCCTCCAGAACTTCTCTGTGTCAAGCCATTTGGCT   1392
OPT  --C--G--T--G-----C--G--T-----GC-------G-----------G--TT-A--------------CAGCTCT---C----C   1392
     P  K  D  I  D  L  T  P  K  E  S  G  I  G  K  I  P  P  T  Y  Q  I  C  F  S  A  R  H  H
ORI  CCCAAGGACATTGACCTCACGCCCAAGGAGAGTGGCATTGGAAAAATACCTCCAACGTACCAGATCTGCTTCTCAGCTCGGCACCAC   1479
OPT  --G-----T------T-A--C--A--A-----C--T--C--C--G--C--G--------------T------AGC--A--C--T--T   1479
     H  H  H
ORI  CACCACCACTAA                                                                              1491
OPT  --------T---                                                                              1491
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